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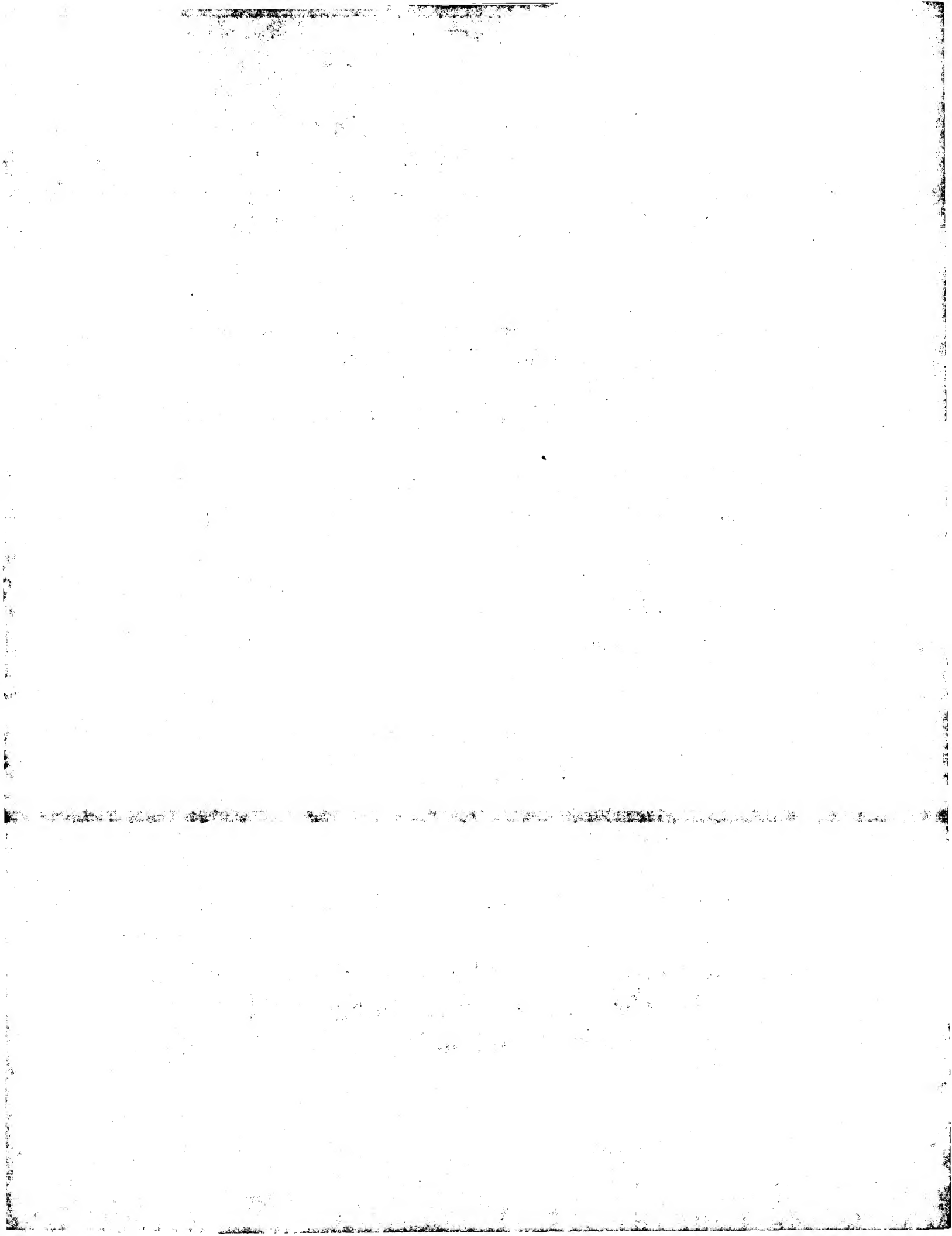
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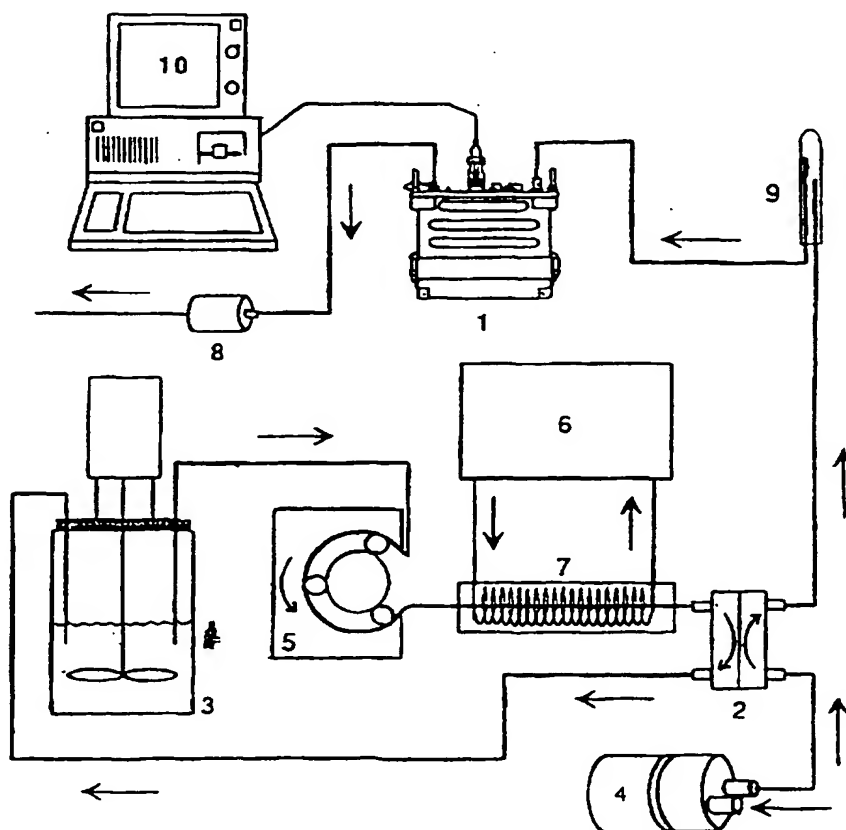
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(54) Title: METHOD FOR MEASUREMENT OF ANALYTES BY ION MOBILITY SPECTROMETRY

(57) Abstract

The invention relates to a method for measurement of process analytes by ion mobility spectrometry. Gas contents are measured in the method and the so obtained gas content values are used to control the progress of a chemical process. An ion mobility spectrometer of aspiration type is used as the instrument.



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METHOD FOR MEASUREMENT OF ANALYTES BY ION MOBILITY SPECTRO-
METRY

The subject invention relates to a method and an instrument
5 for measurement of analytes by ion mobility spectrometry, in
e.g. a fermentation reaction. The objective of the invention
is especially e.g. a direct continuous on-line measurement
of alcohol for control of the alcoholic fermentation, in
e.g. beer and yeast fermentation.

10

Ion mobility spectrometry (IMS) is an analytical method in
which the analyte molecules are ionized by ion/molecule
reactions in a radioactive ion source. The subsequent ions
are separated according to their different mobilities in a
15 weak electrical field at atmospheric pressure. The ion
mobility spectrometry has so far been used for many applica-
tions, such as detection of chemical warfare agents and
drugs and for chromatographic applications.

20 The use of ion mobility spectrometry has been restricted by
its relatively low specificity and limited quantitative
capability. This concerns especially the time-of-flight
method in on-line measurement of analytes.

25 For the measurement of ethanol has previously been used e.g.
gas chromatography, enzymatic methods, membrane inlet mass
spectrometry and electrochemical and solid-state sensors.
Many of these methods have, however, drawbacks, such as long
analysis times in the chromatographic methods, limited life
30 times of the enzymatic sensors, relatively high cost of mass
spectrometers and low specificity and slow response of
sensors.

The sterility requirements involved in bioprocesses hamper
35 or prevent the sampling during the process. This is e.g. the
case in penicillin manufacture, in which fenox acetic acid
is used as the reactant for penicillin. It is essential for
the process that the fenox acetic acid concentration is
within certain limits. The determination of the fenox acetic

acid concentration during the almost two week long fermentation is vital for the yield.

The solution according to the invention provides a considerable improvement of the above mentioned disadvantages. The invention is characterized in what is presented in the claims.

The objective of the invention is to develop a method and an instrument for the measurement of analytes, especially for direct continuous on-line measurement of analytes for process control, which method and instrument are simple, reliable and inexpensive. The objective of the invention is especially to develop a method and an instrument for measurement of alcohols, such as ethanol, and especially for on-line measurement of ethanol by ion mobility spectrometry, preferably by ion mobility spectrometry based on the aspiration method for monitoring fermentation reactions, such as alcoholic and yeast fermentations. The method and instrument according to the invention provide the industry with a fast and inexpensive process control. The instrument according to the invention produces easily and quickly an analysis of several samples and eliminates the separate handling of samples when measuring.

25

The embodiment of the invention offers a quite new and feasible method of measuring analytes with an aspiration-type ion mobility spectrometer. When only one membrane is needed, the gas content does not fall in difficult conditions below the observation limit, which has been the problem when using several membranes. Sampling of the analytes in bioprocesses increases the risk for contamination, and the whole process to be analyzed might even have to be interrupted when taking a sample. Use of a membrane reduces this risk. A continuous on-line measuring method is thus highly desired and requested for bioprocesses and is now embodied with the method and instrument according to the invention.

The method and instrument according to the invention are below described in detail with reference to the enclosed pictures 1-9, in which:

- 5 Fig. 1 is a schematic diagram of the instrument according to the invention installed for measurement of the fermentation process analyte.

Fig. 2a is a picture of the bilobate membrane inlet.

10

Fig 2 b is a cross-section of the Fig. 2a plates.

Fig. 3 presents the signal pattern, embodied by the instrument according to the invention for 2,5% (v/v) ethanol and
15 methanol.

Fig. 4 shows signals of different channels for aqueous solutions of ethanol obtained by the instrument according to the invention.

20

Fig. 5 presents ethanol calibration curves provided by the instrument according to the invention and extracted from the data presented in Fig. 4.

- 25 Fig. 6 presents ethanol calibration curves measured at two different days.

Fig. 7 shows signal patterns of different measurement channels for seven different beer brands.

30

Fig. 8 shows signal patterns received from different detecting channels during yeast fermentation.

- 35 Fig. 9 presents ethanol concentration measurements of Fig. 8 yeast fermentation by membrane mass spectrometry.

The M90-gas detector 3 is an aspiration condenser-type ion mobility spectrometer, in which the air to be analyzed is led through a measuring cell. This device has more in detail been described in the Finnish patent 75055.

5

The M90-gas detector sensor comprises ionization, deflecting and measuring (collecting) regions. M90 uses a radiation source, e.g. a $160\mu\text{Ci } ^{242}\text{AM}$ radioactive ion source for sample ionization. An important feature of the M90-instrument is
10 that it contains separate deflecting and measuring regions for both positive and negative ions, which means that the gas flow can be divided into two separate flows, one for the measurement of positive ions and the other one for the measurement of negative ions. Electrical fields of a prede-
15 termined size transverse to the gas flow are formed in the deflecting and measuring regions with voltage plates. These regions are further divided into three different areas, which enables the simultaneous measurement of positive and negative ions at e.g. six different points, which secures
20 the reliability of the result. Positive ions are measured in their own deflecting and measuring regions and negative ions in their own. Gas to be analyzed is aspirated into a tube, filtered with a heatable filter and led into the ionization cell, which as such can be provided several in parallel or
25 in sequence. The gas is charged by radiation transmitted from e.g. the alpha- or beta-radiation source. The gas is led to a measuring tube. The voltage of the field electrodes in the collecting field is V_1, V_2, \dots, V_n . The back plate voltage is V_r . In the collecting field the light ions charged
30 in the gas are collected into the field electrodes V_n . In the measuring chamber the further advanced remaining heavy ions cause an ion current I_n to the electrodes in the chamber border, which is registered. From each value I_n , in which n is an integer, e.g. 1-6, is formed a diagram, the form of
35 which depicts the substance to be analyzed.

During operation, ambient air and air containing gas to be measured are continuously pumped through the ionization,

deflecting and measuring regions. After ionization of the sample air, part of the ions are collected to the deflection region and part of them are deflected to the measuring electrodes for measurement of the ion current by observing with
5 an electrometer the current caused by the substance to be measured and comparing it with the calibration sample. Due to the continuous operation, the ambient air causes an even-background signal level. Analytes in the sample air cause change in the ion population and therefore a positive or
10 negative change compared to the background signal level is detected, and this change is the sample response.

The M90-instrument can be used alone or it can be connected to a personal computer 10, using a computer program for
15 controlling the device. This program can be used for measurements and to change parameters. The most important parameters which can be changed using this program are collecting electrode gains and flow rate through the instrument. In addition, the program can be used to teach the instrument
20 new compounds, i.e. to measure the characteristic signal pattern for a particular analyte and add the pattern to the library. During this so called standard measurement protocol, 2 signal values per second for each channel are recorded and stored in the computer.

25
The M90-detector type and the continuous signal measurement make it especially suitable for use in the method according to the invention, compared with other ion mobility spectrometers on market, compared e.g. with ion mobility spectrometers provided with a time-of-flight tube, in which the ion
30 mobility is measured against the flow in a reverse electrical field while the current is changing in relation to time.

The building of a suitable interface between the measuring
35 device and the component to be measured in a bioprocess is mainly determined by the nature of the process. In an aerobic process, the medium is bubbled with some proper gas, e.g. synthetic air or nitrogen, typically in the proportion

1:1 (v/v) to the volume of the fermentation chamber. Measuring the component evaporating from the medium from the headspace gas produces information about the component content in the medium itself. The amount of component in the headspace-gas can be affected with a proper mixture. In an anaerobic process, where the medium is not bubbled, the interface must be based on a membrane, whereby the component measured from the medium evaporates through a proper membrane. Temperature of the medium is typically 30 - 40 °C, causing the high relative humidity of the sample gas to create a problem in the first alternative. If determination of the reactant / end product is difficult due to the properties of the measuring device, the state of the process can also be measured indirectly. The bacteria or yeast metabolism yields several other gases or vapors that can be measured. One example is again the fenox acetic acid, which evaporates into the headspace-gas, but seems to contaminate immediately on tubes and measuring chambers, thus complicating the on-line measurement.

In the subject method, the sample is extracted directly from the reaction vessel as shown in Fig. 1, and is pumped with the sample pump 5 to the heat exchanger 7, which is heated with e.g. a water bath 6. From this the sample is led to the membrane inlet 2, which preferably is made of stainless steel. The membrane inlet comprises two plates opposite each other, with spiral grooves 20 provided in the areas coming opposite each other, along which grooves the sample can pass. The width of the grooves is in the example approx. 2 mm and the depth 1 mm (Figs. 2a and 2b). A microporous polypropylene membrane (Celgar 2502, Hoechst Celenase, North-Carolina, USA) is clamped between the plates, and an O-ring is mounted to one of the sides. The Celgard 2502 membrane is 50 µm thick, and has an effective pore size of 0,075 µm and a porosity of 45%. The sample solution is circulated on one side of the membrane and air on the other side. The area of the membrane exposed to the sample solution and to the air is 3 cm². The evaporated analyte con-

tained in the sample solution, which analyte is in a molecule form, passes through the membrane due to diffusion and laminar/turbulent flow interaction (F.R. Lauritsen and D. Lloyd, C. Fenselau (Ed.), "Mass Spectrometry for the Characterization of Microorganisms", ACS Symposium Series 541, American Chemical Society, Washington DC 1994, p. 91). The temperature of both the membrane inlet 2 and the input sample flow is properly regulated for the measuring device. The membrane inlet is surrounded by an insulating material in order to achieve a stable temperature.

As shown, the sample solution is hereafter conducted to one side of the membrane inlet described above, thus transferring the analyte to be measured through the membrane and with the assistance of the air pump 4 to the air stream flowing on the other side of the membrane. The air stream is then led through the water trap 9 to the ion mobility spectrometer. The water trap prevents the humidity from entering the M90-instrument.

20

The experimental part

The operation of the subject method and instrument in monitoring fermentation processes was tested by measuring ethanol concentrations in yeast fermentation. The results of the yeast fermentation were compared with results measured by membrane inlet mass spectrometry.

The described ion mobility spectrometer and membrane inlet as well as other instrument parts were used in the tests. Balzers QMG 420 mass spectrometer was used as membrane inlet mass spectrometer. The special characteristics related to the use of this device have been described more in detail in the publication F.R. Lauritsen, L.T. Nielsen, H. Degn, D. Lloyd and S. Bohatka, Biol. Mass Spectrom. 20(1991)253. The temperature of the sample cell was 25 °C. The concentration of ethanol in yeast fermentation samples was measured using single ion monitoring (ion m/z 31 monitor) and external

standard calibration. The sampling frequency was 2 samples per hour.

Instrumental parameter determination

5

Standard solutions were made adding ethanol (96% (v/v)) or methanol to distilled water. Several beer brands were acquired from store.

- 10 Bakers yeast fermentations were carried out in a 2 liter fermentor at 33°C. Agitation was 400 rpm and working volume of the fermentation broth was 1,6 liters. The initial glucose concentration (D(+)-glucose monohydrate) and the bakers yeast concentration were 62,5 g/l and 12,5 g/l respectively.
- 15 The fermentation medium was distilled water into which 1 g/1,6 l commercial wine/beer fermentation salt mixture (Vinicole A/S, Denmark) was added.

Deflection voltages were determined for ethanol by changing

20 the deflection voltage default values of the M90-gas detector. The deflection voltages can be changed in the range of - 5 - + 5 V using the potentiometers connected to the sensor part of the M90 instrument. Continuous ethanol standard introduction via the sensor provided with a mem-

25 brane was used during the calibration. The results of the calibration are shown in Fig. 3, which presents a signal pattern for the ethanol 2,5%-solution (v/v). The channels 1, 2 and 3 are for positive ions and the channels 4, 5 and 6 are for negative ions. As it can be seen from Fig. 3, there

30 is a clear maximum of the signal at one positive ion channel and one negative ion channel. Fig. 3 displays also a signal pattern for the methanol 2,5%-solution (v/v). When comparing the ethanol and methanol signal patterns, it can be established that the M90 IMS-device can easily separate these two

35 fairly similar chemical substances. It should be noted that the sensitivity of methanol is considerably lower than that of ethanol.

The normal air flow rate through the detector part of the instrument was 2,4 l/min. The effects of the membrane area, of the sample/membrane temperature and of the sample flow rate on the ethanol signal were also studied.

5

All measurements were implemented by using the above mentioned microporous polypropylene membrane, which was selected based on results obtained in the membrane mass spectrometry when analyzing small polar compounds direct
10 from an aqueous sample, F.R. Lauritsen, T.K. Choudhury, L.E. Dejarne and R.G. Cooks, Anal. Chim. Acta, 266 (1992) 1.

The effect of membrane area on the ethanol signal was tested by measuring the ethanol signal of 5 different aqueous
15 ethanol solutions (in the range 0,2 - 5% (v/v)), using two membrane inlets having different active membrane areas, i.e. 1,6 cm² and 3 cm². As expected, the measurements evidenced that a better sensitivity was achieved when using a large active membrane area. A 50% increase of the total signal
20 level was achieved with a larger membrane area than by a smaller membrane area.

The large membrane area was used in the subsequent tests because of its better sensitivity.

25

The effect of the sample/membrane temperature on ethanol signal levels was tested by measuring at three different temperatures (25°C, 35°C and 45°C) using five different ethanol solutions (in the range of 0,2 - 5% (v/v)). It was
30 established that higher temperatures give better sensitivity. Increasing the sample/membrane temperature from 25°C to 45°C, increased the total signal levels about 50%. However, the use of higher temperatures is restricted by the higher moisture content of the sample air flow. The relative humid-
35 ity of the air flow increased from 35% to 67% when the temperature was increased from 25°C to 45°C. The temperature 40°C was selected for all tests to obtain a good sensitivity.

The effect of the sample flow rate on the ethanol signal was tested using several sample flow rates in the range of 4,5 - 48 ml/min. No significant differences in the ethanol signal level were observed. A typical value for sample flow rate 5 was 20 ml/min, and this was used in all measurements. The air flow rate through the membrane inlet was 1,4 l/min. in all measurements. Note that the air pumped through the membrane inlet and the air sucked by the M90 instrument via the water trap was unpurified laboratory air.

10

Results of the ethanol measurements

Fig. 4 shows a typical response of various detector channels to the M90-ion mobility spectrometer calibrated as presented 15 above for aqueous solutions of ethanol at 0,2, 0,5, 1,0, 2,0, 5,0, 7,5 and 10% (v/v) levels, as a function of time. Data in Fig. 4 were obtained by sequentially injecting 30-s injections of ascending ethanol concentrations into the continuous water stream passing through the membrane inlet. 20 Channel 4 signal is not presented in Fig. 4, since it stayed at the constant background level throughout the whole experiment. The very good stability of the signals presented in Fig. 4 for each of the sample solutions illustrates the quantitative reproducibility of the membrane inlet ion 25 mobility instrument.

From Fig. 4 it can be seen that the ethanol response is very fast. Rise times (10-90%) and fall times (90-10%) for various ethanol solutions were in the range of 5-10 s. Typically 30 the rise times were a few second shorter than the fall times. The calibration curves presented in Fig. 5 were extracted from the data shown in Fig. 4. As can be seen from Fig. 5 signal linearity at channels 1 and 3 is relatively good in the whole concentration range, but for the other 35 channels the signal is linear as a function of the ethanol concentration only in very small concentration ranges. Correlation coefficients of 0.989 and 0.999 were calculated for channel 1 and channel 3, respectively. It appears that

the signals of the channels 1 and 3 are preferable for quantitative analysis when external standard calibration is used. However, good signal linearity is not necessarily required for good quantization, since nonlinear calibration curves can be also used for quantization, if the reproducibility of the calibration curves is good. In Fig. 6 calibration curves for channels 1, 3 and 6 measured during two different days are shown, indicating that calibration curves can be relatively well repeated even when the measurement system has been turned completely off and new standard solutions are being prepared for the second measuring. Typically the signal levels could be repeated within $\pm 10\%$ on a day to day basis.

15 Ethanol determination in commercial beers

Fig. 7 shows response of various measurement channels of the M90-instrument for seven different Danish beers. The alcohol content of the beers declared on the labels was 4,6% (v/v). Again the channel 4 signal is not presented since it stayed at the constant background level during the measurement. From Fig. 7 it can be seen that all the beer samples give responses of about the same magnitude, which is a good indication that the M90 IMS instrument can be used for quantitative ethanol measurements. Another indication of quantitative capabilities of membrane inlet ion mobility spectrometry is good reproducibility of signal levels, especially at the channels which give the best response for ethanol. Six times repeated measurements of the beer sample showed that a coefficient of variation value of 1% was obtained for channels 2, 5 and 6 and values of 6 and 10% for channels 3 and 1, respectively.

Ethanol concentrations of the beer samples were calculated based on two different external standard calibrations, one obtained using ethanol standards prepared in distilled water and the other obtained using ethanol standards prepared by adding ethanol to a light beer (original ethanol concentra-

tion 2,6% (v/v), ethanol added to get 5,0 and 7,5% (v/v) solutions). Ethanol concentrations shown in Table 1 were obtained by calculating the average for the 35 points on the height of the sample peak, and by using this average response value to determine the ethanol concentration from the calibration curves obtain as a result of standard measurements. The channel 5 was not used in these calculations since it was very close to the saturation level. The Table 1 results confirm the result observable from Fig. 7, in which the samples 4, 6 and 7 contain more ethanol than the other samples. Ethanol standards prepared in water gave too high alcohol contents from the channel 2, 3 and 6 signals. Alcohol contents of the beer relatively close to the declared contents were obtained only at channel 1. Standard solutions prepared in beer gave good quantitative results at all channels.

Table 1

Beer sample	Channel 1		Channel 2		Channel 3		Channel 6	
	Beer STD	Water STD	Beer STD	Water STD	Beer STD	Water STD	Beer STD	Water STD
1	4.8	4.4	4.8	6.6	4.3	6.3	4.5	6.4
2	4.9	4.5	4.8	6.7	4.3	6.4	4.6	6.4
3	4.5	4.1	4.8	6.7	4.2	6.3	4.5	6.4
4	4.8	4.4	5.1	7.0	4.6	6.7	4.8	6.9
5	4.5	4.1	4.8	6.7	4.4	6.5	4.6	5.4
6	5.1	4.7	5.2	7.1	4.7	7.0	4.9	6.9
7	4.8	4.5	5.0	6.9	4.7	7.0	4.8	6.8

Table 2 presents results of other beer alcohol content measurements using the method developed above. This test was using beer samples with an alcohol content of 4,6 (v/v) (content declared on the bottle), as calibration standard solution. The choice of beer as standard solution is based

on the results presented above, which disclosed that when determining the alcohol content of beer, the determination of the calibration curve using ethanol aqueous solutions give wrong results. From Table 2 it can be seen that the calibration method used gives relatively good results for the lower alcohol content beers, especially when channels 1 and 3 are considered. This result is understandable since the best linearity was observed at channels 1 and 3. The result for the high alcohol content beer is not very good which fact again confirms that the best quantization results will be obtained with a standard which is as similar as possible to the sample solution. Finally it should be noted that there generally are big variations in the alcohol contents declared for the commercial beers (4-5%).

Table 2

Beer sample	Channel 1	Channel 2	Channel 3	Channel 6
1, 2.6 v/v%	2.6	3.4	2.6	3.3
2, 5.9 v/v%	5.4	4.9	5.1	4.9
3, 9.4 v/v%	7.8	6.1	8.9	5.2

Ethanol determination in yeast fermentation

Ethanol production in yeast fermentation was also studied by on-line monitoring of the ethanol concentration by a membrane inlet ion mobility spectrometry. The measuring results are presented in Fig. 8, where the second adding of glucose is marked by arrow. The results in Fig. 8 display that the channel 2 and 6 signals show the expected result, i.e. a relatively constant increase of the signal as the yeast is growing and producing ethanol. The channels 1, 4 and 5 show, however, unexpected results. The channel 1 signal increases at first quickly, is then stabilized for a moment, whereafter it starts decreasing, as could be expected. Channel 4 indicates an even unexpected increase during the

whole test and the channel 5 signal is saturated unexpectedly fast. It is, however, sufficient for the monitoring of the yeast fermentation that the channels 2, 3 and 6 give good results of the ethanol concentration growth. Ethanol concentration growth was also measured by membrane mass spectrometry, and the results are presented in Fig. 9. The results in Fig. 9 confirm the relatively even growth of ethanol concentration indicated by channels 2, 3 and 6. In the Fig. 8 test, glucose (50 g) was added 310 minutes after the starting of the fermentation (position of the arrow in Fig. 8); but no change in ethanol concentration was observed by either spectrometry (Figs. 8 and 9).

The method and instrument according to the invention can naturally be modified by an experienced craftsman within the scope of protection presented in the enclosed claims.

CLAIMS

1. A method of determining gas contents in manufacturing processes, generating, or from which can be separated gas
5 containing analytes, gas introduced from the manufacturing process is ionized, led through a condenser construction, in which has been formed an electrical field essentially vertically in relation to the gas movement direction, and the current formed by gas ions containing analytes is measured,
10 which corresponds to the gas content, c h a r a c t e r i - z e d in that the sample is conducted from the reaction vessel to one side of the membrane inlet (2) containing the membrane, the gas is pumped with the gas pump (4) to the other side of the membrane inlet (2), which gas conveys the
15 reaction solution analyte which passed through the membrane to be measured in the ion mobility spectrometer (1), preferably an aspiration-type ion mobility spectrometer.

2. A method according to claim 1, c h a r a c t e r i z e d
20 in that the gas content is calculated using the change in the background flow signal as sample response based on the gas calibration results.

3. A method according to claims 1 or 2, c h a r a c t e -
25 r i z e d in that the mobility division of the collected gas ions is measured.

4. A method according to one or several of the claims, c h a r a c t e r i z e d in that the temperature of the
30 sample solution is regulated.

5. A method according to one or several of the claims, c h a r a c t e r i z e d in that the measurement of the collected gas ions is performed on-line.

35

6. A method according to one or several of the claims, c h a r a c t e r i z e d in that the air flow containing the collected gas ions introduced from the membrane inlet is

led through the water trap (9) to the ion mobility spectrometer.

7. A method according to one or several of the claims,
5 c h a r a c t e r i z e d in that the air flow rate of the collected gas to the ion mobility spectrometer is 1 - 3 l/min, preferably 2 - 2,5 l/min.

8. A method according to one or several of the claims,
10 c h a r a c t e r i z e d in that the gas evaporating from the process is led directly to the ion mobility spectrometer.

9. A method according to one or several of the claims,
15 c h a r a c t e r i z e d in that the process is a fermentation reaction, alcohol fermentation, yeast or bacteria fermentation, and/or that the analyte is ethanol or other evaporating bioprocess product.

20 10. A method according to one or several of the claims, c h a r a c t e r i z e d in that gas containing the analyte is in the analyzing device divided into at least two essentially similar flows, from which the content of the analyte contained in the gas is measured.

25

11. A method for measurement of analytes in bioprocesses, in which the analyte to be measured is led through a membrane, having one side in contact with the aqueous sample and the other side with the ambient substance, to the analyte
30 measuring device, and in which method a constant flow is led through the device, c h a r a c t e r i z e d in that with the ion mobility spectrometer
a) the reaction solution is led from the reaction vessel via the heat exchanger (7) to one side of a bilobate membrane
35 inlet (2) containing a microporous membrane, which membrane inlet, constructed from stainless steel, contains discoid stainless steel plates (11a, 11b) keeping the membrane in

between them, which plates are provided with rectangular spiral grooves,

- b) air is pumped with the air pump (4) to the other side of the membrane inlet, which air transports the reaction solution analyte passed through the membrane to the ion mobility spectrometer (1),
- c) the analyte content is measured with the ion mobility spectrometer (1) using the change in the background flow signal as the sample signal,
- 10 d) the analyte content is calculated based on the calibration results of the analyte.

12. An instrument for on-line measurement of analytes in bioprocesses, characterized in that it comprises:

- a) a sample pump (5) for introduction of the reaction solution from the reaction vessel through the heat exchanger (7) to the membrane inlet,
- b) a bilobate membrane device (2), comprising discoid stainless plates (11a, 11b) in a stainless steel vessel, which plates have rectangular spiral grooves, on one side of which membrane device (2) circulates the reaction solution and on the other side the air stream,
- c) a microporous membrane, clamped between the mentioned plates, which membrane has been chosen so that the analyte to be measured penetrates it and enters into the air stream,
- d) an ion mobility spectrometer (1), to which constantly is conducted an air stream from the membrane (2), which spectrometer measures ionization radiation of the background flow in an on-line operation and the change in the background flow forms the sample signal.

13. An instrument according to claim 12, characterized in that the width of the mentioned spiral grooves is 2 mm and the depth 1 mm.

14. An instrument according to claims 11-12, characterized -

t e r i z e d in that the microporous membrane is a polypropylene membrane, and that the effective area of the membrane is 3 cm².

- 5 15. An instrument according to claim 14, c h a r a c t e -
r i z e d in that the thickness of the membrane is 50 μm
and the porosity is 45%.

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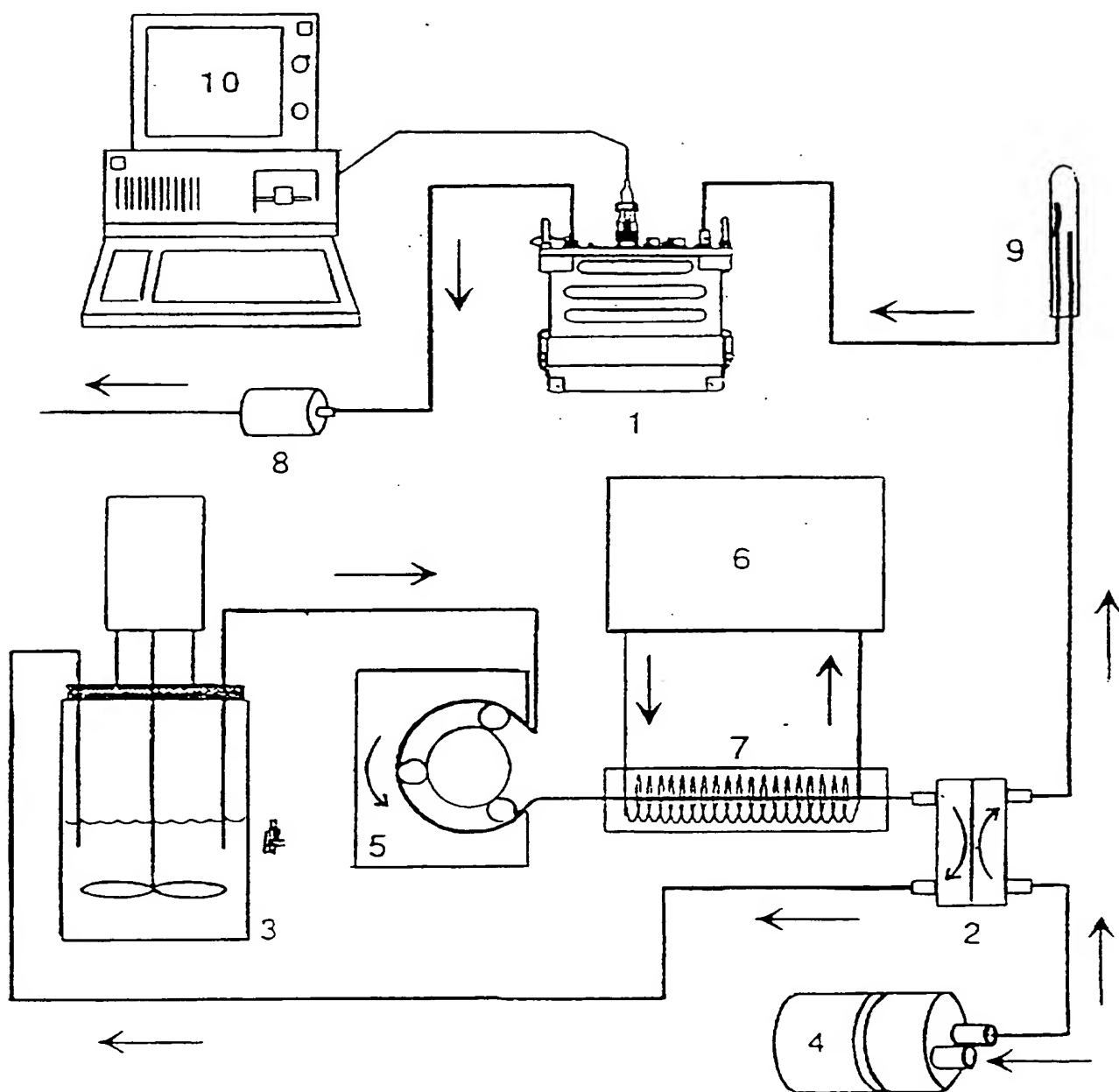


FIG. 1

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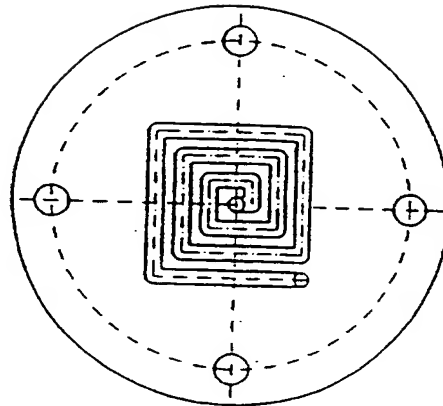


FIG. 2a

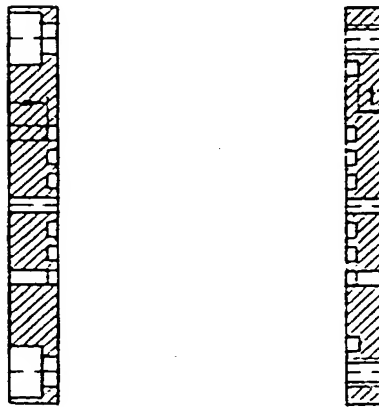


FIG. 2b

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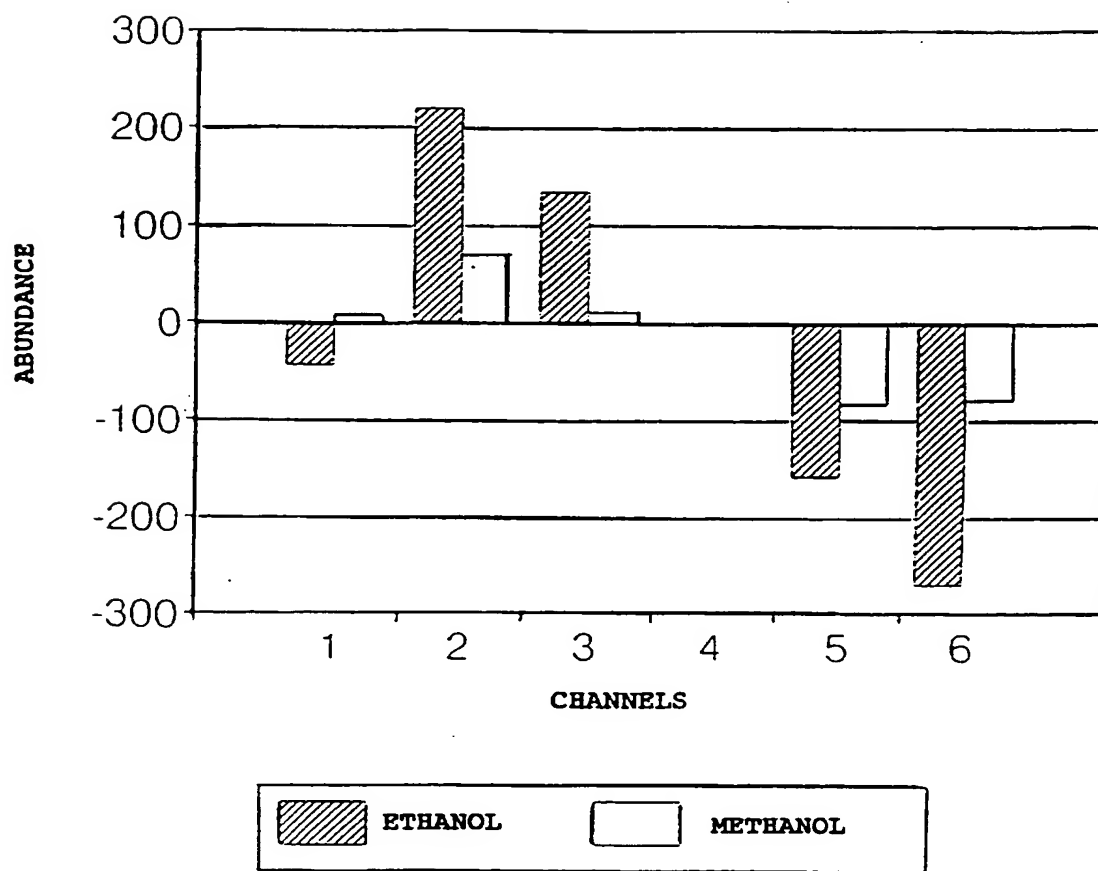


FIG. 3

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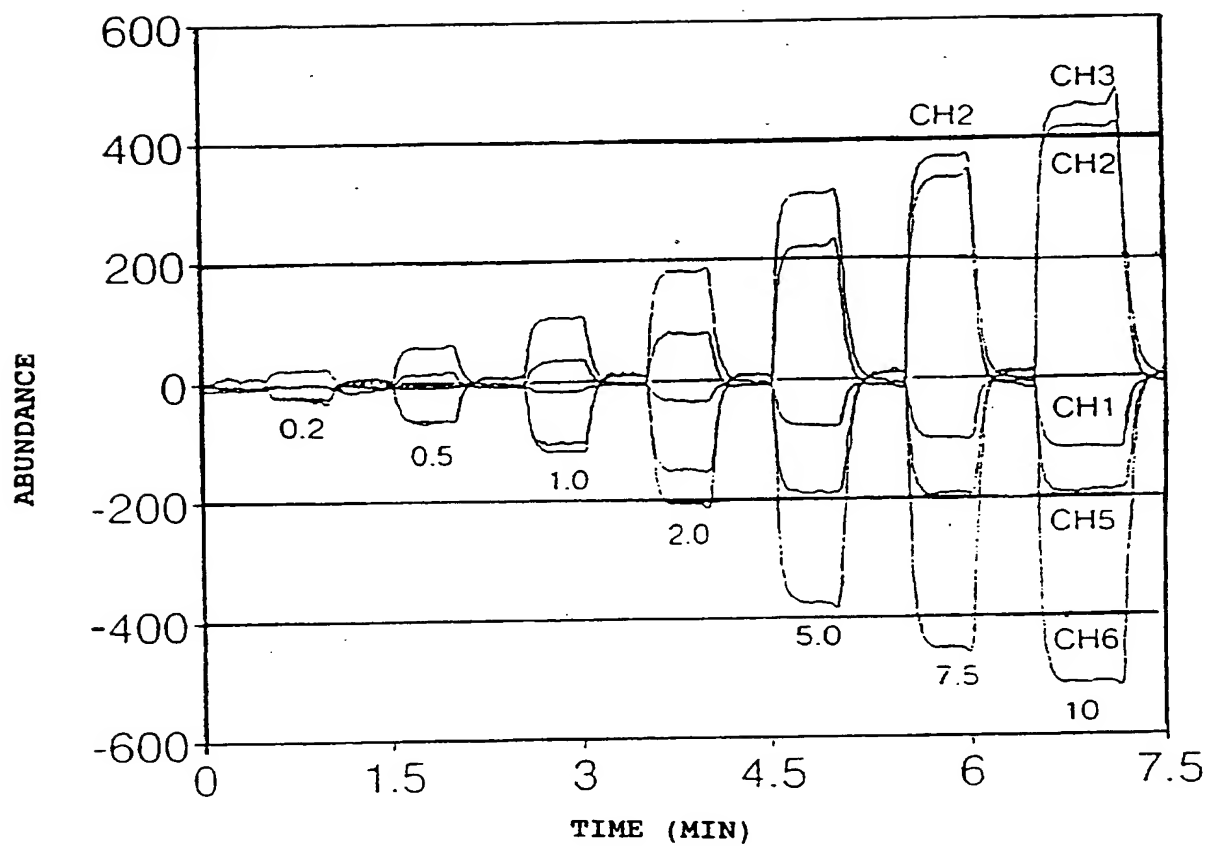


FIG. 4

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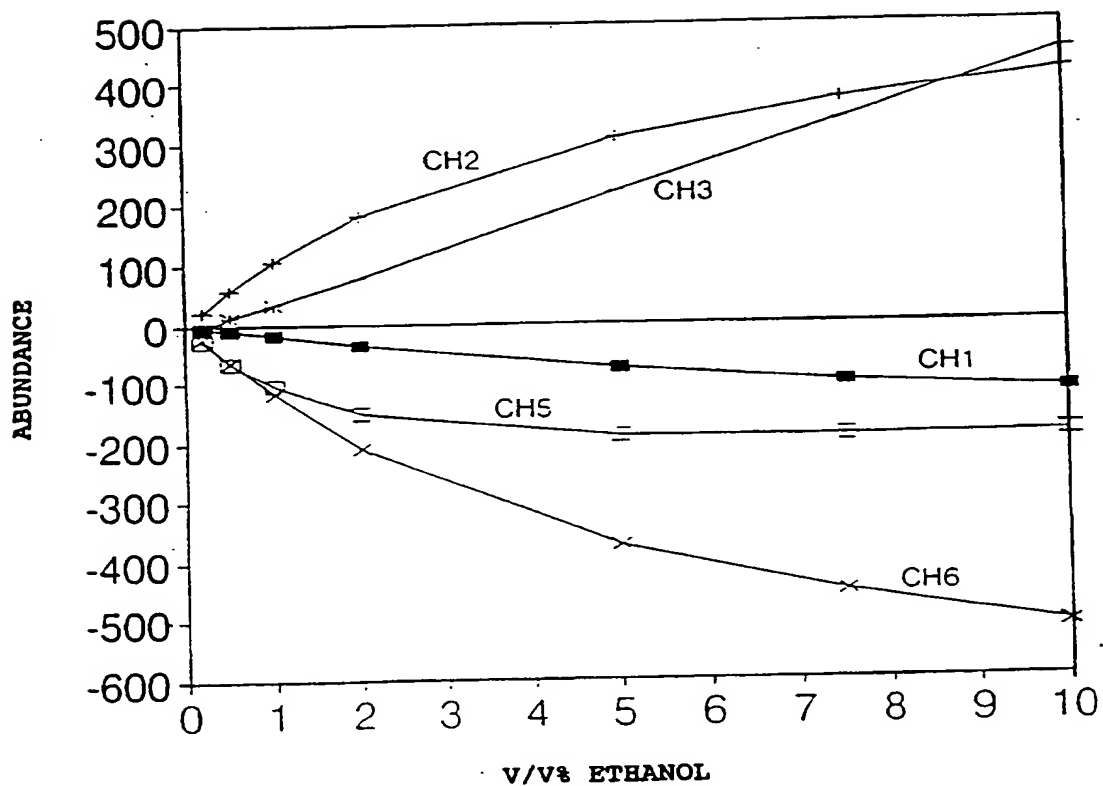


FIG. 5

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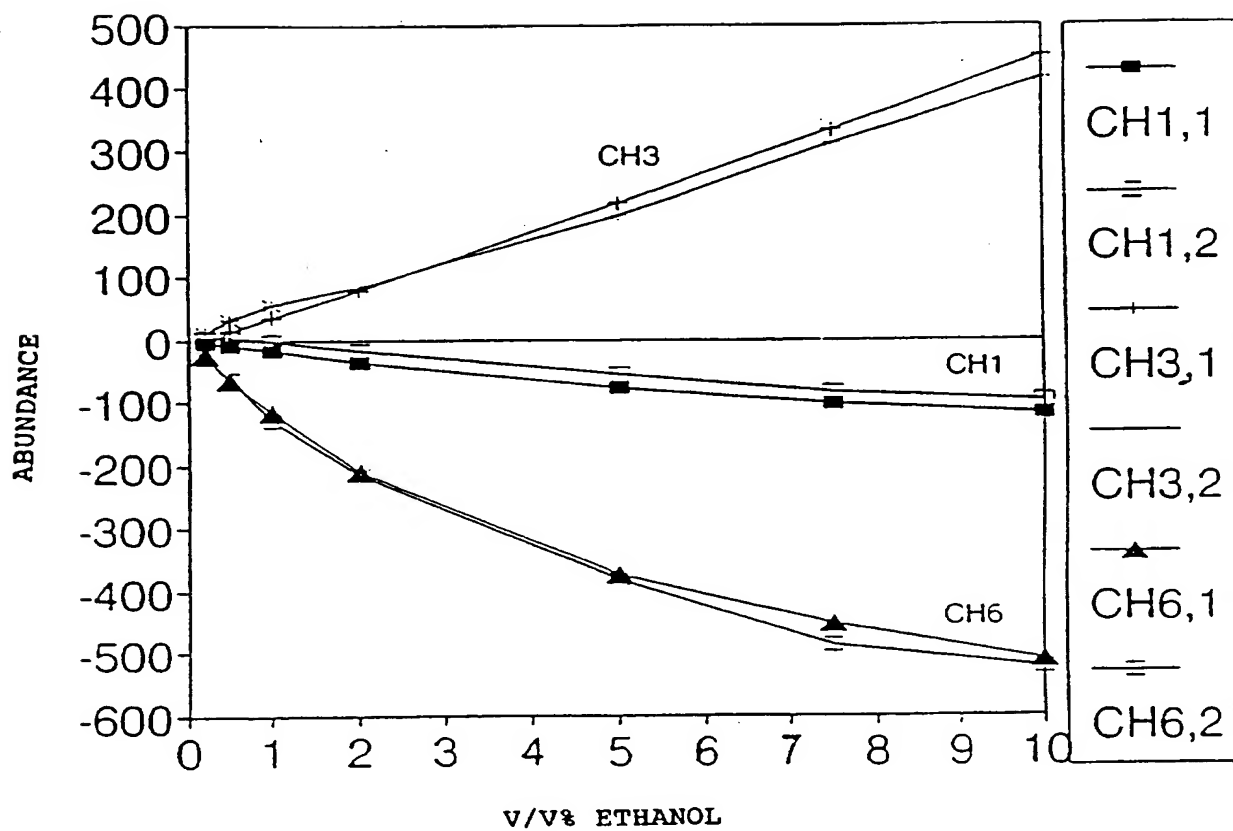


FIG. 6

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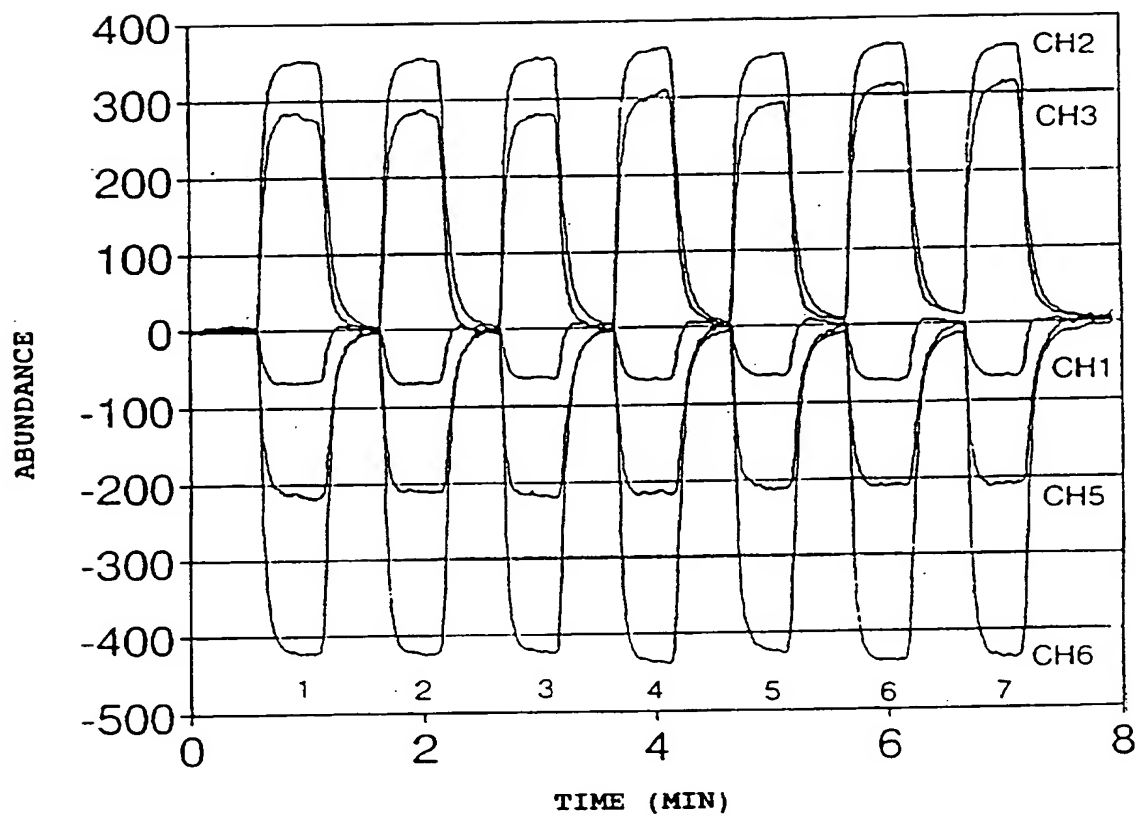


FIG. 7

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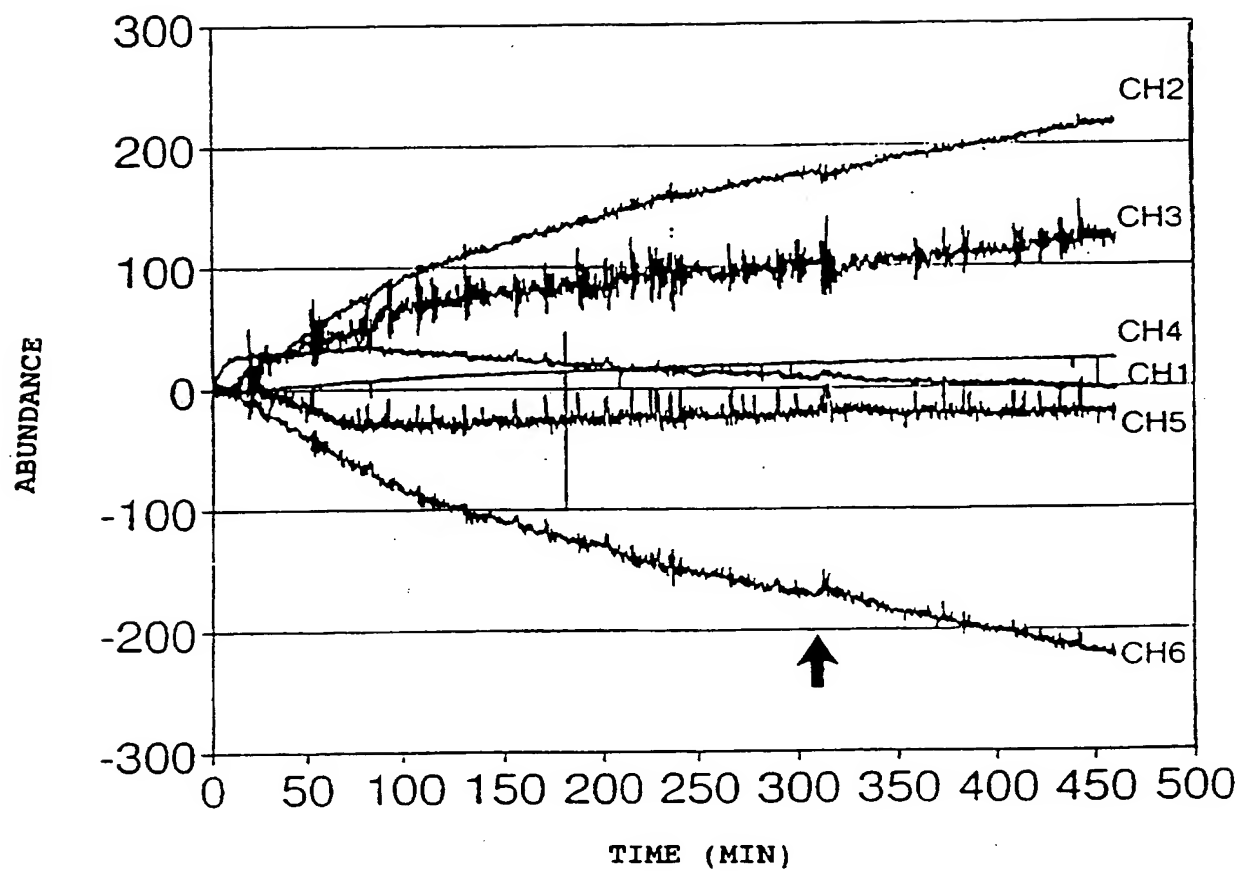


FIG. 8

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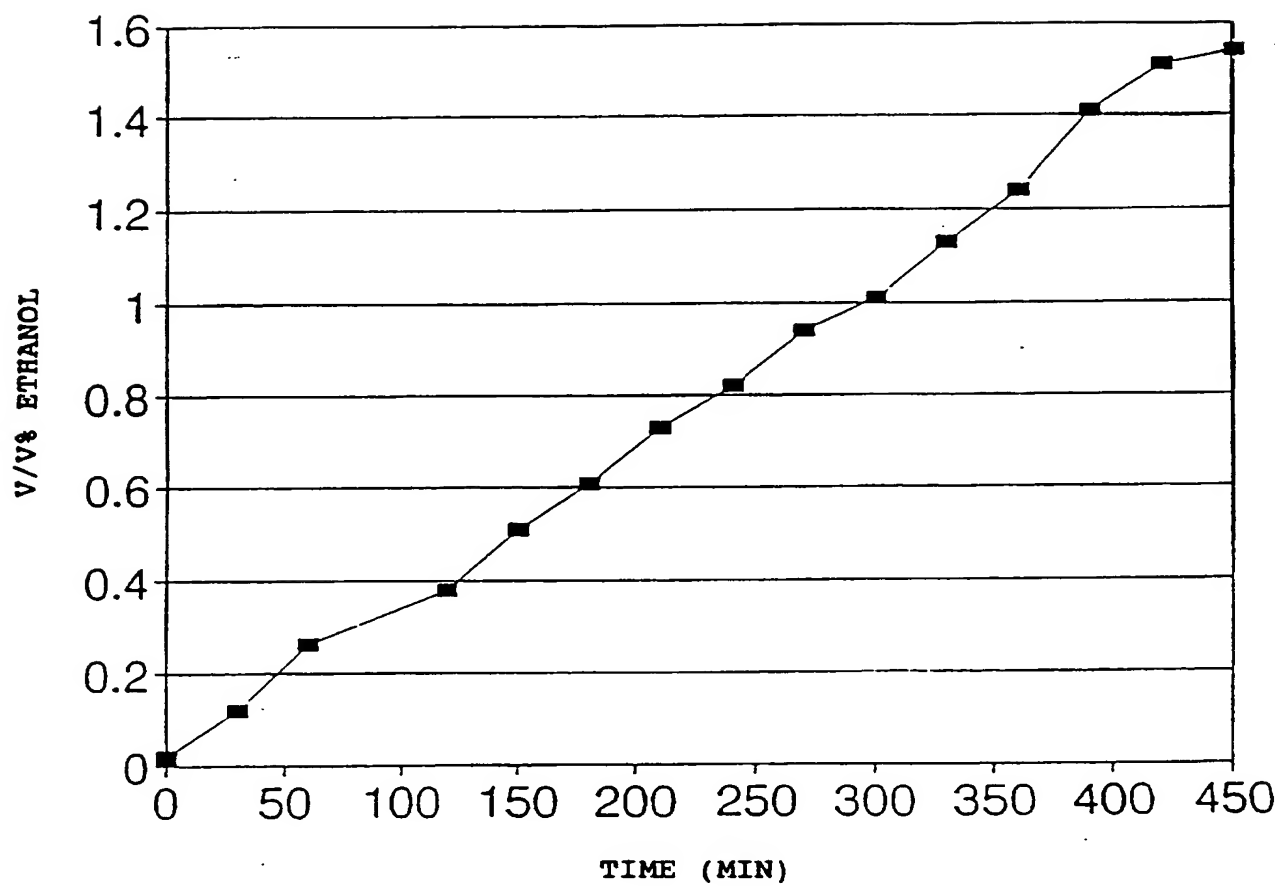


FIG. 9

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 95/00684

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 27/64

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9009583 A1 (GRASEBY IONICS LIMITED), 23 August 1990 (23.08.90), page 1 - page 3; page 16, line 11 - line 27, figure 1, claims 1,6, abstract	1-10
A	--	11-15
A	WO 9207255 A1 (PUUMALAINEN CONSULTS OY), 30 April 1992 (30.04.92), figure 1, claims 1-5, abstract	1-15

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search

13 March 1996

Date of mailing of the international search report

15 -03- 1996

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INTERNATIONAL SEARCH REPORT
Information on patent family members

05/02/96

International application No.
PCT/FI 95/00684

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		EP-A,A- 0626579	30/11/94
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		CA-A- 2093657	12/04/92
		EP-A- 0552206	28/07/93
		JP-T- 6507472	25/08/94

